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Matrigel Invasion Assay Protocol

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An abbreviated version of this protocol was published in eLIFE in Feb 2020

Derivation of trophoblast stem cells from naïve human pluripotent stem cells

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Detailed protocol

Materials and Reagents

1. EVTs or hTSCs
2. EVT basal medium [DMEM/F12 (Gibco, 11320) supplemented with 0.1 mM b-mercaptoethanol (Millipore Sigma, 8.05740), 0.5% penicillin-streptomycin (Gibco, 15140), 0.3% BSA (Gibco, 15260), 1% ITS-X (Gibco, 51500), 7.5 mM A83-01 (BioVision, 1725), 2.5 mM Y27632 (Stemgent, 040012)]
3. TSC medium [DMEM/F12 supplemented with 0.1 mM 2-mercaptoethanol, 0.2% FBS, 0.5% Penicillin-Streptomycin, 0.3% BSA, 1% ITS-X, 1.5 mg/ml L-ascorbic acid (Wako, 013–12061), 50 ng/ml EGF (Rockland, 009–001 C26), 2 mM CHIR99021 (Stemgent, 04–0004), 0.5 mM A83-01, 1 mM SB431542 (BioVision, 1674), 0.8 mM VPA (Tocris, 2815), and 5 mM Y-27632]
4. Fetal bovine serum (FBS) (Millipore Sigma, ES-009-B)
5. TrypLE Express (Gibco, 12604)
6. Matrigel-coated transwell inserts with 8.0µm pores (Corning, 354480)
7. 4% PFA
8. Cotton tipped swab
9. PBS

Equipment

1. Centrifuges
2. Humidified tissue culture incubator, 37^{O}C, 5% CO_2
3. Microscope

Procedure

1. *Preparation of trans-well inserts*

a. Place desired number of matrigel-coated inserts into a 24-well companion plate and allow them to come to room temperature.
b. Add warm (37^{O}C) bicarbonate based culture medium to the interior of the inserts and bottom of wells. Allow the inserts to rehydrate for 2 hours in a humidified tissue culture incubator.
c. After rehydration, carefully remove the medium without disturbing the layer of Corning® Matrigel® Matrix on the membrane. Don't let the Matrigel dry, so make sure the cells are ready when the Matrigel is rehydrating.

2. Prepare cells (Upper Chamber)

a. Rinse EVTs or hTSCs once with 10 mL PBS; add 3 mL of TrypLE Express and incubate at 37^{O}C for 5 min; neutralize the trypsin with FBS containing EVT/TSC medium.
b. In a 50 mL conical tube, centrifuge cells at 300 ×g for 10 min.
c. Count cells using a hemocytometer.
d. Prepare 2.0 × 10^5 cells in 200 µL of EVT basal medium or TSC medium.
e. Cap tube and store at room temperature until they are ready to be loaded in the upper chamber of transwell inserts.

3. Prepare the chemoattractant (For Bottom Chamber)

a. For the bottom chamber, prepare 800 µL of EVT basal medium or TSC medium supplemented with 20% FBS for each well.
b. Add 800 µL of chemoattractant carefully to each well. Avoid creating bubbles.

4. Assemble the Invasion Chamber

a. Using sterile forceps, carefully transfer the Matrigel-coated inserts to the wells containing chemoattractant. Make sure there are no air bubbles trapped beneath the insert. If there are bubbles, remove insert and try again.
b. Add 200 µL of cell suspension (preparation as described above) to the upper chamber of Matrigel-coated inserts.
c. Incubate the inserts at 37°C, 5% CO_2 for 36 h for Cell Invasion Assay.

5. Measurement of cell invasion

a. After invasion period, carefully aspirate the media from the insert without disturbing the Matrigel layer and wash twice with PBS.
b. Fix inserts with 500 µL of 4% paraformaldehyde for 15 min in an empty well.
c. Remove fixing solution and wash twice with PBS.
d. Add 500 µL of crystal-violet solution to the empty wells and place the inserts over it for 15 min to stain the cells.
e. Remove inserts from the crystal-violet solution and rinse inserts in three small beakers containing PBS to remove the extra stain.
f. Remove the non-invaded cells on the upper chamber carefully by scrubbing with a cotton tipped swab. Repeat the scrubbing with a second swab moistened with medium.
g. Place the insert on a slide and count the invasive cells under a microscope and capture the images.

References

1. Angelova M, Zwezdaryk K, Ferris M, Shan B, Morris CA, Sullivan DE. Human cytomegalovirus infection dysregulates the canonical Wnt/beta-catenin signaling pathway. PLoS Pathog. 2012; 8(10):e1002959. [PubMed: 23071438].
2. Warner JA, Zwezdaryk KJ, Day B, Sullivan DE, Pridjian G, Morris CA. Human cytomegalovirus infection inhibits CXCL12- mediated migration and invasion of human extravillous cytotrophoblasts. Virol J. 2012; 9:255. [PubMed: 23116176]
3. Zhang L, Wang K, Wu Q, Jin L, Lu H, Shi Y, Liu L, Yang L, Lv L. Let-7 inhibits the migration and invasion of extravillous trophoblast cell via targeting MDM4. Molecular and cellular probes. 2019 Jun 1;45:48-56.

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1. Dong, C. , Popli, P. , Kommagani, R. and Theunissen, T. W.(2021). Matrigel Invasion Assay Protocol. Bio-protocol Preprint. bio-protocol.org/prep793.
2. Dong, C., Beltcheva, M., Gontarz, P., Zhang, B., Popli, P., Fischer, L. A., Khan, S. A., Park, K., Yoon, E., Xing, X., Kommagani, R., Wang, T., Solnica-Krezel, L. and Theunissen, T. W.(2020). Derivation of trophoblast stem cells from naïve human pluripotent stem cells. eLIFE. DOI: 10.7554/eLife.52504